TRANSGENIC ANIMAL AND METHODS

PRIORITY

[0001] This application claims priority to Provisional U.S. Patent Application entitled, "Transgenic Animal and Methods," filed Jul. 13, 2000, having a serial No. 60/218,054, which is hereby incorporated in its entirety by reference.

SUPPORT

[0002] This invention was developed with support from the National Institute of Health, Grant No. AG09665, and the U.S. Government may therefore have certain rights in this invention.

1. FIELD OF THE INVENTION

[0003] This invention relates to a transgenic animal model of Alzheimer's disease and related neurological disorders in which the animal harbors a transgene encoding a protease inhibitor such as antichymotrypsin (ACT) protein. The invention further relates to transgenic animal models of Alzheimer's disease and related neurological disorders in which the animal harbors a transgene encoding a protease inhibitor such as antichymotrypsin (ACT) protein and one or more further transgenes affecting said neurological disorders. The invention further relates to cells comprising a transgene encoding an antichymotrypsin (ACT) protein. The invention also relates to drug screening assays using the invented transgenic cells or transgenic animals and progeny thereof.

2. BACKGROUND OF THE INVENTION

[0004] Biochemical, genetic, and epidemiological evidence indicates that inflammation is an essential part of the pathogenesis of Alzheimer's disease. For example, several acute phase/inflammatory molecules in the brain, specifically antichymotrypsin (ACT) and apolipoprotein E (apoE) can promote the formation of the neurotoxic amyloid deposits that are the main pathological hallmark of the disease. For further details and background information on Alzheimer's disease and related neurological diseases see, for example, U.S. Pat. Nos. 5,297,562;

See Examiner's Amendment

[0023] FIG. 1 Illustrates expression of human ACT; Left panel shows Northern blot hybridization with an ACT probe of polyA+ mRNA from (lane 1) untransfected and (lane 2) GFAP-HACT DNA, and (lane 3) untreated and (lane 4) IL-1 treated U373 MG human astrocytoma cells showing the position of the native human ACT transcript (which is slightly smaller than the fusion gene transcript); Two Right Panels show Immunoprecipitation/Western blot showing ACT protein in (lane 1-2) transfected, (lanes 3-4) untransfected C6 glioma cells. Untransfected cell spiked with 10 pg and 1 ng respectively of human ACT are shown in lanes 5, 6. Lanes 7 and 8 (and a shorter exposure of lanes 9 and 10) show cells transfected with a CMV-ACT construct which expresses ACT at high levels.

DS 4/7/04 **[0024]** FIG. 2 A-F illustrates expression of ACT mRNA and protein in ACT transgenic mice. Immunoprecipitation/Western blot of brain protein extracts from a non-transgenic mouse and ACT-transgenic founder lines (#8782, #8783 and #8784) displaying a protein band (~68 kDa) that comigrates with human serum ACT (**a**). GFAP/ACT-mRNA and GAPDH-mRNA expression in brain of non-transgenic mouse and various tissues of heterozygous ACT-transgenic mouse (**b**). Colocalization of ACT (brown) and GFAP (blue)-immunoreactivity in astrocytes of $ACT^{+/-}$-mice (**c**). Profound astrocyte expression and secretion of ACT-immunoreactivity in the hippocampal formation of heterozygous ACT-transgenic mice (**d**) but absence of ACT-immunostaining in non-transgenic mouse (**e**) 3 days after stab wound injury. Sections were counterstained with Methyl Green (**d** and **e**).

DS 4/7/04 **[0025]** FIG. 3 A-F Illustrates astrocyte-specific expression and plaque association of ACT protein in ACT transgenic mice. ACT-immunopositive astrocytes in a 10 months old PDGF-$hAPP(V717F)^{+/-}$, $ACT^{+/-}$-mice (**a**), but absence of immunostaining in a 10 month old PDGF-$hAPP(V717F)^{+/-}$-$ACT^{+/-}$-mice (**b**). The astrogliotic ACT-immunostaining was clearly visible along the hippocampal fissure in 6 months old mice (**c**). High-power magnification of ACT-immunopositive Congo-positive amyloid plaque in a PDGF-$hAPP(V717F)^{+/-}$, $ACT^{+/-}$-m- ice at 10 months of age (**d**), displaying birefringence under polarized light (**e**).

[0026] FIG. 4 Illustrates increased plaque load and density in APP/ACT transgenic mice. Increased total Congo-positive amyloid load (**a**) and numerical plaque density (**b**) in the